



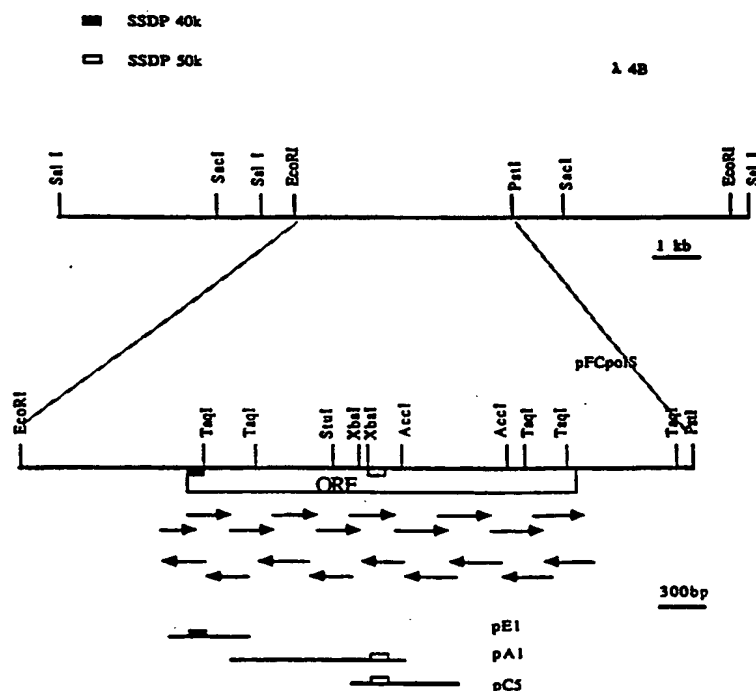
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<b>(21) International Application Number:</b> PCT/IT93/00058 <b>(22) International Filing Date:</b> 10 June 1993 (10.06.93) <b>(30) Priority data:</b> RM92A000438 11 June 1992 (11.06.92) IT <b>(71) Applicant (for all designated States except US):</b> CONSIGLIO NAZIONALE DELLE RICERCHE [IT/IT]; P.le Aldo Moro, 7, I-00185 Rome (IT). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> PISANI, Francesca, Maria [IT/IT]; ROSSI, Mosé [IT/IT]; Istituto di Biochimica delle Proteine ed Enzimologia, Via Marconi, 10, I-80125 Napoli (IT). DE ROSA, Mario [IT/IT]; Istituto Biochimica delle Macromolecole, Ia Fac. Medicina e Chirurgia, Università di Napoli, Via Costantinopoli, 16, I-Napoli (IT).		<b>(74) Agents:</b> BANCHETTI, Marina et al.; Ing. Barzanò & Zanardo Roma S.p.A., Via Piemonte, 26, I-00187 Rome (IT).  <b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	

**(54) Title:** NUCLEOTIDE SEQUENCES CODING FOR A THERMOSTABLE DNA POLYMERASE, DNA POLYMERASE AND USES THEREOF



**(57) Abstract**

Nucleotide sequences coding a polypeptide or fragments thereof having a thermostable and thermophilic DNA polymerase activity, preferably derived from DNA of bacteria of *Sulfolobus* genus, DNA polymerase and uses thereof.

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NUCLEOTIDE SEQUENCES CODING FOR A THERMOSTABLE DNA  
POLYMERASE, DNA POLYMERASE AND USES THEREOF

SPECIFICATION

5 The present invention concerns the isolation  
and the identification of sequences coding a DNA  
polymerase from bacteria belonging to the *Archaea*  
domain (Woese C.R. et al. 1990, Proc. Natl.  
Acad.Sci. USA 87, 4576-4579), to the protein coded  
by said sequence and to uses thereof.

10 DNA polymerases are enzymes responsible of  
the duplication of genomic DNA and, therefore, of  
the inheritance of the genetic material. Sequences  
coding DNA polymerase from bacteria belonging to  
the *Archaea* domain are not known in the prior art.  
15 Such bacteria are adapted to grow at high  
temperatures, and are evolutionary far from  
*Eubacteria*.

DNA polymerases may be classified in two  
classes (Ito, J., and Braithwaite, D.K. (1991)  
20 Nucleic Acids Res. 19, 4045-4057). Class A  
comprises dideoxynucleotide inhibition sensitive  
and aphidicolin resistant enzymes, as pol I from *E.*  
*coli* (Joyce, C.M., Kelley, W.S., and Grindley, N.D.  
F. (1982) J. Biol. Chem. 257, 1958-1964); class B  
25 is more heterogeneous, comprising aphidicolin  
sensitive and partially dideoxynucleotide  
inhibition resistant enzymes.

The authors of the instant invention have  
demonstrated that DNA polymerase extracted from  
30 bacteria of thermostable and thermophilic *Sulfolobus*  
*solfataricus* species has a molecular weight of  
around 100 kDa, by means of gel filtration  
chromatography and of glycerol gradient  
centrifugation. An electrophoresis in denaturing

conditions on polyacrylamide gel shows, other than the 100 kDa protein, two major bands, respectively of 50 e 40 kDa. These bands represent proteolytic cleavage fragments of the 100 kDa protein, being  
5 able to react with antisera raised against the native 100 kDa protein. Moreover the 50 kDa fragment keeps a DNA polymerase activity (Karawya, E., Swack, J.A., and Wilson, S.H. (1983) Anal. Biochem. 135, 318-325).

10 The authors of the present invention have isolated and sequenced the gene coding the DNA polymerase from *S. solfataricus*, and have deduced the aminoacid sequence of the protein. Upon  
15 insertion into procaryotic or eucaryotic expression vectors and transformation of suitable hosts, the gene makes possible the production through recombinant DNA techniques of the DNA polymerase enzyme.

According to the invention the term  
20 "thermofilic" refers to enzymes with a peak of activity at temperatures comprised between 50°C and 85°C, preferably 75°C, when a substrate of DNA from activated calf thymus is used; the term  
"thermostable" refers to the fact that the enzyme  
25 keeps 100% of activity after incubation for 40 min at 75°C.

It is an object of the invention a nucleic acid of natural, recombinant or synthetic origin, comprising a nucleotide sequence coding a  
30 polypeptide or fragments thereof having a thermostable and thermofilic DNA polymerase activity. Preferably said nucleotide sequence is derived from DNA of bacteria of the Archaeadomain, preferably of the *Sulfolobus* genus, more preferably  
35 of the *S. solfataricus* species.

In a preferred embodiment said polypeptide or fragments thereof have also a 3'-5' exonuclease activity.

5 Preferably said nucleotide sequence codes the polypeptide having the aminoacid sequence of SEQ ID N2, or fragments thereof, alternatively deleted or substituted for one or more aminoacids, so that said DNA polymerase activity is maintained.

10 Further object of the invention is a nucleic acid comprised in the sequence of SEQ ID N1 characterized in that from nucleotide 1 to nucleotide 197 is a non coding sequence, from nucleotide 198 to nucleotide 2843 coding a polypeptide with a thermostable and thermophilic DNA  
15 polymerase activity and from nucleotide 2844 to nucleotide 3112 is a non coding sequence. Alternatively said nucleotide sequence lacks or is substituted of one or more nucleotides so that said DNA polymerase activity is maintained.

20 Another object of the invention are nucleotide sequences able to hybridize at medium stringency to nucleotide sequences of the invention, preferably said sequences are complementary to the sequences of the invention.

25 It is another object of the invention a polypeptide with a thermostable and thermophilic DNA polymerase activity, preferably produced through recombinant DNA techniques by nucleotide sequences according to the invention, preferably by the  
30 nucleotide sequence comprised in SEQ ID N1.

According to the invention said polypeptide has a sequence comprised in SEQ ID N2.

It is a further object of the invention recombinant cloning or expression vectors, having a  
35 plasmid or viral derivation, comprising the

nucleotide sequences of the invention, preferably said vector is the plasmid pFCpols (DSM N.7091).

Another object are cells transformed with said vectors.

5           The invention will be described in the following examples, with reference to the following figures:

figure 1 which represents a restriction map of the coding region of the DNA polymerase gene of  
10 *S. solfataricus*;

figures 2a and 2b which represent a sequence analysis of DNA polymerase sequences from different organisms.

Example 1 Partial aminoacid sequence of DNA  
15 polymerase from *S. solfataricus*

30 µg of DNA polymerase purified from *S. solfataricus*, as described in Rossi M. et al. 1986, System. Appl. Microbiol. 7, 337-341, is loaded on a 10% polyacrylamide gel in denaturing conditions.  
20 The gel is then electro-transferred on a PVDF membrane (Problott, Applied Biosystems), as described in Matsudaira, P. (1987) J. Biol. Chem. 262, 10035-10038. The membrane is stained with Coomassie Brilliant Blue R-250. Three protein bands  
25 of 100, 50 e 40 kDa are cutted and loaded directly on a gas-phase aminoacid sequencer (M. 470 A, Applied Biosystems), with an analyzer PTH 120 A. N-terminal sequences of 50 e 40 kDa peptides are:

50 kDa	GYKGAVVIDP
30    40 kDa	SAPVEEKKVVR

Example 2 Isolation and sequence of the DNA  
polymerase gene of *S. solfataricus*

By using aminoacid sequences the following degenerated oligonucleotides are synthesized:

35           29-mer SSDP50K corresponding to the N-terminal sequence of the 50 kDa fragment:

5'-GGATA(T/C) GG(T/A) GG(T/A) GC(T/A)  
GT(T/A) GT(T/A) AT(T/A) GAT CC-3'

23-mer SSDP40K corresponding to the N-terminal sequence of the 40 kDa fragment:

5 5'-GC(T/A) CC(T/A) GT(T/A) GA(A/G) AA(A/G)  
AA(A/G) GT-3'.

Each oligonucleotide is labelled at its 5' end with  $\gamma$ P<sup>32</sup>ATP by means of T4 polynucleotide kinase and used to screen a genomic library of *S. solfataricus*, strain MT4 (ATCC n. 49155), in the  $\lambda$  gtl1vector, at the EcoRI site, according to standard methods. Filter hybridization are made at 45°C with the SSDP40K probe and at 50°C with the SSDP50K probe, in 6 x saline citrate buffer (SSC) as described in Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) in Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor. Inserts of positive phages pA1, pC5 e pE1 are subcloned into the EcoRI site of the pUC18 vector, and sequenced (Sequenase, USB). The inserts have partial overlapping regions and an open reading frame, as shown in Fig. 1.

Another genomic library obtained in the  $\lambda$  EMBL3 vector with MboI partially digested DNA of *S. solfataricus*, producing fragments of around 15 Kb, according to standard methods. The library is screened with the EcoRI insert of pC5 clone as probe. Hybridizations are performed on filters at 65°C, 6 x SSC, according to Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) in Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor. Two positive phages  $\lambda$  4B and  $\lambda$  2P are purified and digested with restriction enzymes (Fig. 1). The EcoRI-PstI fragment, present in both phages, and able to hybridize with pE1, pA1 and pC5 clones is inserted

into the pEMBL8 vector, producing the plasmid named pFCpolS (DSM N. 7091). The sequence is shown in SEQ ID N1. The sequence shows a region of 882 codons with an open reading frame, in agreement with the 100 kDa molecular weight of the protein. The 5' end non coding region does not comprise promoter sequences homologous to other Archaeabacterial promoters (Reiter, W.D., Palm, P., and Zillig, W. (1988) Nucleic Acids Res. 16, 1-19; Reiter, W.D., Hudepohl, U., and Zillig, W. (1990) Proc. Natl. Acad. Sci. USA 87, 9509-9513. A pyrimidine rich region comprising the TTTTAT sequence is present at the 3' end of the termination codon, in analogy with other terminators from Archaea bacteria (Cubellis, M.V., Rozzo, C., Nitti, G., Arnone, M.I., Marino, G., and Sannia, G. (1989) Eur. J. Biochem. 186, 375-381; Cubellis, M.V., Rozzo, C., Montecucchi, P., and Rossi, M. (1990) Gene 94, 89-94; Reiter, W.D., Palm, P., and Zillig, W. (1989) Nucleic Acid Res. 16, 2445-2459).

Example 3 Sequence homology with other DNA polymerases

A sequence analysis shows homologies with class B DNA polymerases, as viral eucaryote replicases (Gibbs, J.S., Chiou, H.C., Hall, J.D., Mount, D.W., Retondo, M.J., Weller, S.K., and Coen, D.M. (1985) Proc. Natl. Acad. Sci. USA 82, 7969-7973; Kouzarides, T., Bankier, A.T., Satchwell, S.C., Weston, K., Tomlison, P., and Barrel, B.G. (1987) J. Virol. 61, 125-133; Earl, P.L., Jones, E.V., and Moss, B. (1986) Proc. Natl. Acad. Sci. USA 83, 3659-3663), human replicases (Wong, S.W., Wahl, A.F., Yuan, P.M., Arai, N., Pearson, B. E., Arai, K.-I., Korn, D., Hunkapiller, M.W., and Wang, T. S.-F. (1988) EMBO J. 7, 37-47) and DNA polymerase  $\alpha$  of *S. cerevisiae* (Pizzagalli, A.,



Valsasnini, P., Plevani, P., and Lucchini, G. (1988) Proc. Natl. Acad. Sci. USA 85, 3772-3776). Few homologies are evident with *E. coli* DNA polymerases (Joyce, C.M., Kelley, W.S., and Grindley, N.D. F. (1982) J. Biol. Chem. 257, 1958-1964).

Class B DNA polymerases show conserved motifs (Ito, J., and Braithwaite, D.K. (1991) Nucleic Acids Res. 19, 4045-4057; Wong, S.W., Zahl, A.F., Yuan, P.-M., Arai, N., Pearson, B. E., Arai, K.-I., Korn, D., Hunkapiller, M.W., and Wang, T. S.-F. (1988) EMBO J. 7, 37-47; Iwasaki, H., Ishino, Y., Toh, H., Nakata, A., and Shinagawa, H. (1991) Mol. Gen. Genet. 226, 24-33; Larder, B.A., Kemp, S.D., and Darby, G. (1987) EMBO J. 6, 169-175; Bernard, A., Zaballo, A., Salas, M., and Blanco, L. (1987) EMBO J. 6, 4219-4225; Blanco, L., Bernard, A., Blasco, M.A., and Salas, M. (1991) Gene 100, 27-38), which are found also in the sequence of the invention, as shown in Figs. 2a and 2b, regions 1-8.

Regions 1, 2 e 3 correspond to EXO motifs found in DNA polymerases with 3'-5' exonuclease activity (Morrison, A., Bell, J.B., Kunkel, T.A., and Sugino, A. (1991) Proc. Natl. Acad. Sci. USA 88, 9473-9477), where three aspartic acid and one glutamic acid residues are maintained.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: Consiglio Nazionale delle Ricerche
- (B) STREET: P.le Aldo Moro 5
- (C) CITY: Roma
- (D) STATE: Italy
- (E) COUNTRY: Italy
- (F) POSTAL CODE (ZIP): 00185

(ii) TITLE OF INVENTION: Nucleotide sequences coding for a DNA polymerase, DNA polymerase and uses thereof

(iii) NUMBER OF SEQUENCES: 2

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3112 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Sulfolobus solfataricus*

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 198..2846

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATCTGGTGTT TTTCTTTCTC ATGCATATTATAAATGTTTA CTAAGATTCA AGGCATATCT 60
CTTAAGAAAT GGCTAGATGA ATGAGAGGAG CAGGAGTAGC TTAAGAATCT TAAAACTTAG 120
GTTCTTCATA AATGTCTATT TTTTCTCCCG CATTAAAACT TATAGCGTAT TTCTCAGAAA 180
ATAATATATG TTAGAAA ATG ACT AAG CAA CTT ACC TTA TTT GAT ATT CCT 230
      Met Thr Lys Gln Leu Thr Leu Phe Asp Ile Pro
              1              5              10

TCA TCT AAA CCC GCT AAG AGT GAA CAA AAT ACT CAA CAA TCG CAA CAG 278
Ser Ser Lys Pro Ala Lys Ser Glu Gln Asn Thr Gln Gln Ser Gln Gln
              15              20              25

AGT GCT CCC GTT GAG GAA AAA AAG GTA GTT AGG AGG GAA TGG CTT GAA 326
Ser Ala Pro Val Glu Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu
              30              35              40

GAG GCT CAG GAA AAT AAG ATA TAC TTC CTA TTG CAA GTA GAT TAT GAT 374
Glu Ala Gln Glu Asn Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp
              45              50              55

GGT AAG AAA GGT AAG GCT GTA TGT AAG CTA TTC GAT AAA GAA ACT CAA 422
Gly Lys Lys Gly Lys Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln
              60              65              70              75

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AAG ATC TAT GCC CTA TAT GAT AAT ACT GGA CAT AAG CCC TAC TTT CTA	470
Lys Ile Tyr Ala Leu Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu	
80 85 90	
GTA GAT CTT GAA CCT GAT AAA GTA GGT AAA ATA CCT AAG ATT GTT AGA	518
Val Asp Leu Glu Pro Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg	
95 100 105	
GAT CCA TCT TTT GAT CAC ATA GAG ACT GTG AGT AAG ATA GAC CCG TAT	566
Asp Pro Ser Phe Asp His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr	
110 115 120	
ACT TGG AAT AAA TTC AAA TTA ACT AAA ATC GTT GTT AGA GAT CCC CAT	614
Thr Trp Asn Lys Phe Lys Leu Thr Lys Ile Val Val Arg Asp Pro His	
125 130 135	
GCA GTG AGA AGA TTA AGG AAT GAT GTT CCA AAA GCG TAT GAG GCT CAC	662
Ala Val Arg Arg Leu Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His	
140 145 150 155	
ATA AAA TAT TTT AAC AAC TAC ATG TAT GAC ATA GGT CTA ATC CCC GGT	710
Ile Lys Tyr Phe Asn Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly	
160 165 170	
ATG CCT TAT GTT GTT AAG AAT GGG AAG TTA GAA AGT GTC TAT TTG TCT	758
Met Pro Tyr Val Val Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser	
175 180 185	
TTG GAC GAG AAA GAT GTT GAG GAG ATT AAG AAA GCC TTC GCT GAT TCA	806
Leu Asp Glu Lys Asp Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser	
190 195 200	
GAT GAA ATG ACT AGA CAA ATG GCA GTC GAT TGG CTT CCC ATA TTT GAA	854
Asp Glu Met Thr Arg Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu	
205 210 215	



GCA GTG AGG AAT TAT GCA TTT GAG GGA AAG TAT AAT GAA TAC AAT TTA	1334
Ala Val Arg Asn Tyr Ala Phe Glu Gly Lys Tyr Asn Glu Tyr Asn Leu	
365 370 375	
GAT GCA GTT GCA AAG GCC TTA TTA GGG ACA TCA AAA GTT AAG GTA GAT	1382
Asp Ala Val Ala Lys Ala Leu Leu Gly Thr Ser Lys Val Lys Val Asp	
380 385 390 395	
ACG CTA ATA TCT TTC TTA GAT GTA GAA AAA TTA ATA GAA TAT AAC TTT	1430
Thr Leu Ile Ser Phe Leu Asp Val Glu Lys Leu Ile Glu Tyr Asn Phe	
400 405 410	
AGG GAT GCC GAA ATC ACA CTT CAG CTT ACT ACA TTT AAT AAC GAC CTA	1478
Arg Asp Ala Glu Ile Thr Leu Gln Leu Thr Thr Phe Asn Asn Asp Leu	
415 420 425	
ACT ATG AAG TTA ATT GTA TTG TTT TCT AGA ATT TCT AGA CTA GGA ATT	1526
Thr Met Lys Leu Ile Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile	
430 435 440	
GAG GAA TTA ACT CGG ACA GAA ATA TCT ACT TGG GTA AAG AAT TTA TAT	1574
Glu Glu Leu Thr Arg Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr	
445 450 455	
TAT TGG GAA CAT AGA AAA AGA AAT TGG TTA ATT CCT CTT AAG GAA GAA	1622
Tyr Trp Glu His Arg Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu	
460 465 470 475	
ATC TTA GCG AAA TCC TCT AAT ATA AGA ACT TCT GCT CTA ATA AAG GGA	1670
Ile Leu Ala Lys Ser Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly	
480 485 490	
AAA GGA TAT AAA GGC GCA GTA GTT ATA GAC CCA CCT GCT GGA ATA TTC	1718
Lys Gly Tyr Lys Gly Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe	
495 500 505	

TTT AAC ATA ACT GTT TTA GAT TTT GCA TCA CTA TAT CCT TCA ATA ATT	1766
Phe Asn Ile Thr Val Leu Asp Phe Ala Ser Leu Tyr Pro Ser Ile Ile	
510 515 520	
AGA ACG TGG AAT CTT AGT TAC GAG ACT GTA GAC ATT CAA CAA TGT AAG	1814
Arg Thr Trp Asn Leu Ser Tyr Glu Thr Val Asp Ile Gln Gln Cys Lys	
525 530 535	
AAG CCC TAT GAA GTA AAG GAT GAG ACA GGG GAG GTG CTA CAT ATA GTT	1862
Lys Pro Tyr Glu Val Lys Asp Glu Thr Gly Glu Val Leu His Ile Val	
540 545 550 555	
TGC ATG GAT AGG CCA GGT ATA ACA GCA GTA ATA ACT GGG TTA CTA AGA	1910
Cys Met Asp Arg Pro Gly Ile Thr Ala Val Ile Thr Gly Leu Leu Arg	
560 565 570	
GAC TTC AGA GTA AAG ATA TAC AAA AAG AAA GCG AAG AAC CCT AAT AAT	1958
Asp Phe Arg Val Lys Ile Tyr Lys Lys Lys Ala Lys Asn Pro Asn Asn	
575 580 585	
AGT GAG GAA CAA AAA CTA CTC TAT GAC GTA GTA CAG AGA GCA ATG AAA	2006
Ser Glu Glu Gln Lys Leu Leu Tyr Asp Val Val Gln Arg Ala Met Lys	
590 595 600	
GTA TTC ATA AAT GCT ACT TAC GGT GTA TTT GGA GCT GAA ACA TTT CCG	2054
Val Phe Ile Asn Ala Thr Tyr Gly Val Phe Gly Ala Glu Thr Phe Pro	
605 610 615	
TTA TAT GCG CCA CGT GTA GCG GAG AGT GTT ACT GCA CTG GGG AGA TAC	2102
Leu Tyr Ala Pro Arg Val Ala Glu Ser Val Thr Ala Leu Gly Arg Tyr	
620 625 630 635	
GTT ATT ACC AGT ACC GTA AAG AAA GCT AGG GAA GAA GGT TTA ACT GTA	2150
Val Ile Thr Ser Thr Val Lys Lys Ala Arg Glu Glu Gly Leu Thr Val	
640 645 650	

TTA TAC GGT GAT ACT GAT TCT TTA TTC CTC CTT AAT CCT CCC AAG AAT	2198
Leu Tyr Gly Asp Thr Asp Ser Leu Phe Leu Leu Asn Pro Pro Lys Asn	
655 660 665	
AGT TTA GAA AAT ATT ATA AAA TGG GTT AAA ACT ACT TTC AAT TTA GAT	2246
Ser Leu Glu Asn Ile Ile Lys Trp Val Lys Thr Thr Phe Asn Leu Asp	
670 675 680	
TTG GAA GTT GAT AAA ACC TAC AAG TTT GTG GCT TTT TCT GGA TTG AAG	2294
Leu Glu Val Asp Lys Thr Tyr Lys Phe Val Ala Phe Ser Gly Leu Lys	
685 690 695	
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Lys Asn Tyr Phe Gly Val Tyr Gln Asp Gly Lys Val Asp Ile Lys Gly	
700 705 710 715	
ATG TTA GTG AAG AAG AGA AAC ACG CCG GAA TTT GTA AAG AAG GTA TTT	2390
Met Leu Val Lys Lys Arg Asn Thr Pro Glu Phe Val Lys Lys Val Phe	
720 725 730	
AAC GAG GTA AAG GAG CTA ATG ATC TCC ATA AAC TCG CCA AAC GAT GTG	2438
Asn Glu Val Lys Glu Leu Met Ile Ser Ile Asn Ser Pro Asn Asp Val	
735 740 745	
AAG GAG ATT AAA AGA AAA ATT GTA GAC GTA GTT AAA GGA TCA TAT GAA	2486
Lys Glu Ile Lys Arg Lys Ile Val Asp Val Val Lys Gly Ser Tyr Glu	
750 755 760	
AAA CTA AAA AAC AAA GGA TAC AAT CTG GAC GAA TTA GCG TTT AAA GTA	2534
Lys Leu Lys Asn Lys Gly Tyr Asn Leu Asp Glu Leu Ala Phe Lys Val	
765 770 775	
ATG CTA TCG AAG CCT TTA GAT GCG TAC AAA AAG AAC ACT CCC CAA CAC	2582
Met Leu Ser Lys Pro Leu Asp Ala Tyr Lys Lys Asn Thr Pro Gln His	
780 785 790 795	



GTA AAG GCA GCT CTA CAA CTT AGA CCA TTT GGA GTT AAC GTA TTA CCA 2630  
 Val Lys Ala Ala Leu Gln Leu Arg Pro Phe Gly Val Asn Val Leu Pro  
 800 805 810

CGA GAT ATA ATA TAC TAT GTT AAG GTT AGA TCT AAA GAT GGA GTG AAA 2678  
 Arg Asp Ile Ile Tyr Tyr Val Lys Val Arg Ser Lys Asp Gly Val Lys  
 815 820 825

CCA GTA CAA CTA GCT AAA GTT ACT GAA ATA GAC GCA GAG AAA TAT TTA 2726  
 Pro Val Gln Leu Ala Lys Val Thr Glu Ile Asp Ala Glu Lys Tyr Leu  
 830 835 840

GAA GCG TTA AGA AGT ACG TTT GAG CAA ATC TTA AGG GCA TTC GGA GTC 2774  
 Glu Ala Leu Arg Ser Thr Phe Glu Gln Ile Leu Arg Ala Phe Gly Val  
 845 850 855

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 Ser Trp Asp Glu Ile Ala Ala Thr Met Ser Ile Asp Ser Phe Phe Ser  
 860 865 870 875

TAC CCA AGT AAA GGA AAT AGT TAATTAAGAA AGATAGCAAT TCTTCATAAT 2873  
 Tyr Pro Ser Lys Gly Asn Ser  
 880

AAATTTT TAG AAGCAATTTT TACCCACATA AGTTATAAAG ATTTT TAGAA AATTTAAATC 2933

GTATATTTTT ATTCTTCCTC CTCTTCCTCT AATTCTTCCT TTAATTCTTC TTGTTTCTGC 2993

ATACCCAAGT AAAGGAAATA GTTAATTAAG AAAGATAGCA ATTCTTCATA ATAAATTTTT 3053

AGAAGCAATT TTTACCCACA TAAGTTATAA AGATTTT TAG AAAATTTAAA TCGTATATT 3112

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 882 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Thr	Lys	Gln	Leu	Thr	Leu	Phe	Asp	Ile	Pro	Ser	Ser	Lys	Pro	Ala
1				5					10					15	
Lys	Ser	Glu	Gln	Asn	Thr	Gln	Gln	Ser	Gln	Gln	Ser	Ala	Pro	Val	Glu
			20					25					30		
Glu	Lys	Lys	Val	Val	Arg	Arg	Glu	Trp	Leu	Glu	Glu	Ala	Gln	Glu	Asn
		35					40					45			
Lys	Ile	Tyr	Phe	Leu	Leu	Gln	Val	Asp	Tyr	Asp	Gly	Lys	Lys	Gly	Lys
	50					55					60				
Ala	Val	Cys	Lys	Leu	Phe	Asp	Lys	Glu	Thr	Gln	Lys	Ile	Tyr	Ala	Leu
65					70					75					80
Tyr	Asp	Asn	Thr	Gly	His	Lys	Pro	Tyr	Phe	Leu	Val	Asp	Leu	Glu	Pro
				85					90					95	
Asp	Lys	Val	Gly	Lys	Ile	Pro	Lys	Ile	Val	Arg	Asp	Pro	Ser	Phe	Asp
			100					105					110		
His	Ile	Glu	Thr	Val	Ser	Lys	Ile	Asp	Pro	Tyr	Thr	Trp	Asn	Lys	Phe
			115					120				125			

Lys Leu Thr Lys Ile Val Val Arg Asp Pro His Ala Val Arg Arg Leu  
 130 135 140

Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn  
 145 150 155 160

Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly Met Pro Tyr Val Val  
 165 170 175

Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser Leu Asp Glu Lys Asp  
 180 185 190

Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser Asp Glu Met Thr Arg  
 195 200 205

Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu Thr Glu Ile Pro Lys  
 210 215 220

Ile Lys Arg Val Ala Ile Asp Ile Glu Val Tyr Thr Pro Val Lys Gly  
 225 230 235 240

Arg Ile Pro Asp Ser Gln Lys Ala Glu Phe Pro Ile Ile Ser Ile Ala  
 245 250 255

Leu Ala Gly Ser Asp Gly Leu Lys Lys Val Leu Val Leu Asn Arg Asn  
 260 265 270

Asp Val Asn Glu Gly Ser Val Lys Leu Asp Gly Ile Ser Val Glu Arg  
 275 280 285

Phe Asn Thr Glu Tyr Glu Leu Leu Gly Arg Phe Phe Asp Ile Leu Leu  
 290 295 300

Glu Tyr Pro Ile Val Leu Thr Phe Asn Gly Asp Asp Phe Asp Leu Pro  
 305 310 315 320

Tyr Ile Tyr Phe Arg Ala Leu Lys Leu Gly Tyr Phe Pro Glu Glu Ile  
325 330 335

Pro Ile Asp Val Ala Gly Lys Asp Glu Ala Lys Tyr Leu Ala Gly Leu  
340 345 350

His Ile Asp Leu Tyr Lys Phe Phe Phe Asn Lys Ala Val Arg Asn Tyr  
355 360 365

Ala Phe Glu Gly Lys Tyr Asn Glu Tyr Asn Leu Asp Ala Val Ala Lys  
370 375 380

Ala Leu Leu Gly Thr Ser Lys Val Lys Val Asp Thr Leu Ile Ser Phe  
385 390 395 400

Leu Asp Val Glu Lys Leu Ile Glu Tyr Asn Phe Arg Asp Ala Glu Ile  
405 410 415

Thr Leu Gln Leu Thr Thr Phe Asn Asn Asp Leu Thr Met Lys Leu Ile  
420 425 430

Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile Glu Glu Leu Thr Arg  
435 440 445

Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr Tyr Trp Glu His Arg  
450 455 460

Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu Ile Leu Ala Lys Ser  
465 470 475 480

Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly Lys Gly Tyr Lys Gly  
485 490 495

Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe Phe Asn Ile Thr Val  
500 505 510

Leu Asp Phe Ala Ser Leu Tyr Pro Ser Ile Ile Arg Thr Trp Asn Leu  
 515 520 525

Ser Tyr Glu Thr Val Asp Ile Gln Gln Cys Lys Lys Pro Tyr Glu Val  
 530 535 540

Lys Asp Glu Thr Gly Glu Val Leu His Ile Val Cys Met Asp Arg Pro  
 545 550 555 560

Gly Ile Thr Ala Val Ile Thr Gly Leu Leu Arg Asp Phe Arg Val Lys  
 565 570 575

Ile Tyr Lys Lys Lys Ala Lys Asn Pro Asn Asn Ser Glu Glu Gln Lys  
 580 585 590

Leu Leu Tyr Asp Val Val Gln Arg Ala Met Lys Val Phe Ile Asn Ala  
 595 600 605

Thr Tyr Gly Val Phe Gly Ala Glu Thr Phe Pro Leu Tyr Ala Pro Arg  
 610 615 620

Val Ala Glu Ser Val Thr Ala Leu Gly Arg Tyr Val Ile Thr Ser Thr  
 625 630 635 640

Val Lys Lys Ala Arg Glu Glu Gly Leu Thr Val Leu Tyr Gly Asp Thr  
 645 650 655

Asp Ser Leu Phe Leu Leu Asn Pro Pro Lys Asn Ser Leu Glu Asn Ile  
 660 665 670

Ile Lys Trp Val Lys Thr Thr Phe Asn Leu Asp Leu Glu Val Asp Lys  
 675 680 685

Thr Tyr Lys Phe Val Ala Phe Ser Gly Leu Lys Lys Asn Tyr Phe Gly  
 690 695 700

Val Tyr Gln Asp Gly Lys Val Asp Ile Lys Gly Met Leu Val Lys Lys  
705 710 715 720

Arg Asn Thr Pro Glu Phe Val Lys Lys Val Phe Asn Glu Val Lys Glu  
725 730 735

Leu Met Ile Ser Ile Asn Ser Pro Asn Asp Val Lys Glu Ile Lys Arg  
740 745 750

Lys Ile Val Asp Val Val Lys Gly Ser Tyr Glu Lys Leu Lys Asn Lys  
755 760 765

Gly Tyr Asn Leu Asp Glu Leu Ala Phe Lys Val Met Leu Ser Lys Pro  
770 775 780

Leu Asp Ala Tyr Lys Lys Asn Thr Pro Gln His Val Lys Ala Ala Leu  
785 790 795 800

Gln Leu Arg Pro Phe Gly Val Asn Val Leu Pro Arg Asp Ile Ile Tyr  
805 810 815

Tyr Val Lys Val Arg Ser Lys Asp Gly Val Lys Pro Val Gln Leu Ala  
820 825 830

Lys Val Thr Glu Ile Asp Ala Glu Lys Tyr Leu Glu Ala Leu Arg Ser  
835 840 845

Thr Phe Glu Gln Ile Leu Arg Ala Phe Gly Val Ser Trp Asp Glu Ile  
850 855 860

Ala Ala Thr Met Ser Ile Asp Ser Phe Phe Ser Tyr Pro Ser Lys Gly  
865 870 875 880

Asn Ser

## CLAIMS

1. Nucleic acid of natural, recombinant or synthetic origin, comprising a nucleotide sequence coding a polypeptide or fragments thereof having a thermostable and thermophilic DNA polymerase activity.

2. Nucleic acid according to Claim 1 wherein said nucleotide sequence is derived from bacteria of the Archaeadomain.

3. Nucleic acid according to Claim 2 wherein said nucleotide sequence is derived from bacteria of the *Sulfolobus* genus.

4. Nucleic acid according to Claim 2 wherein said nucleotide sequence is derived from bacteria of the *S. solfataricus* species.

5. Nucleic acid according to any of previous Claims wherein said polypeptide or fragments thereof have also a 3'-5' exonuclease activity.

6. Nucleic acid according to Claim 5 wherein said nucleotide sequence codes the polypeptide having the aminoacid sequence of SEQ ID N2 or fragments thereof.

7. Nucleic acid according to Claim 6 wherein said nucleotide sequence codes the polypeptide having the aminoacid sequence of SEQ ID N2 or fragments thereof, deleted or substituted for one or more aminoacids, so that said DNA polymerase activity is maintained.

8. Nucleic acid comprised in the sequence of SEQ ID N1 characterized in that from nucleotide 1 to nucleotide 197 is a non coding sequence, from nucleotide 198 to nucleotide 2843 coding a polypeptide with a thermostable and thermophilic DNA polymerase activity and from nucleotide 2844 to nucleotide 3112 is a non coding sequence.

9. Nucleic acid according to Claim 8 wherein said coding sequence lacks or is substituted of one or more nucleotides so that said DNA polymerase activity is maintained.

5           10. Nucleic acid able to hybridize at least at medium stringency to a nucleic acid according to any of previous Claims.

10           11. Nucleic acid according to Claim 10 complementary to nucleotide sequences from Claim 1 to 9.

          12. Polypeptide with a thermostable and thermophilic DNA polymerase activity.

15           13. Polypeptide according to Claim 12 produced through recombinant DNA techniques by nucleic acids according to any of previous Claims from 1 to 11.

          14. Polypeptide according to Claim 13 produced by the nucleotide sequence comprised in SEQ ID N1.

20           15. Polypeptide according to Claim 14 having a sequence comprised in SEQ ID N2.

25           16. Recombinant cloning or expression vectors, having a plasmid or viral derivation, comprising nucleotide sequences according to any of previous Claims from 1 to 11.

          17. Recombinant vector according to Claim 16 being the plasmid pFCpolS (DSM N.7091).

          18. Cells transformed with vectors according to Claims 16 or 17.



FIG. 1

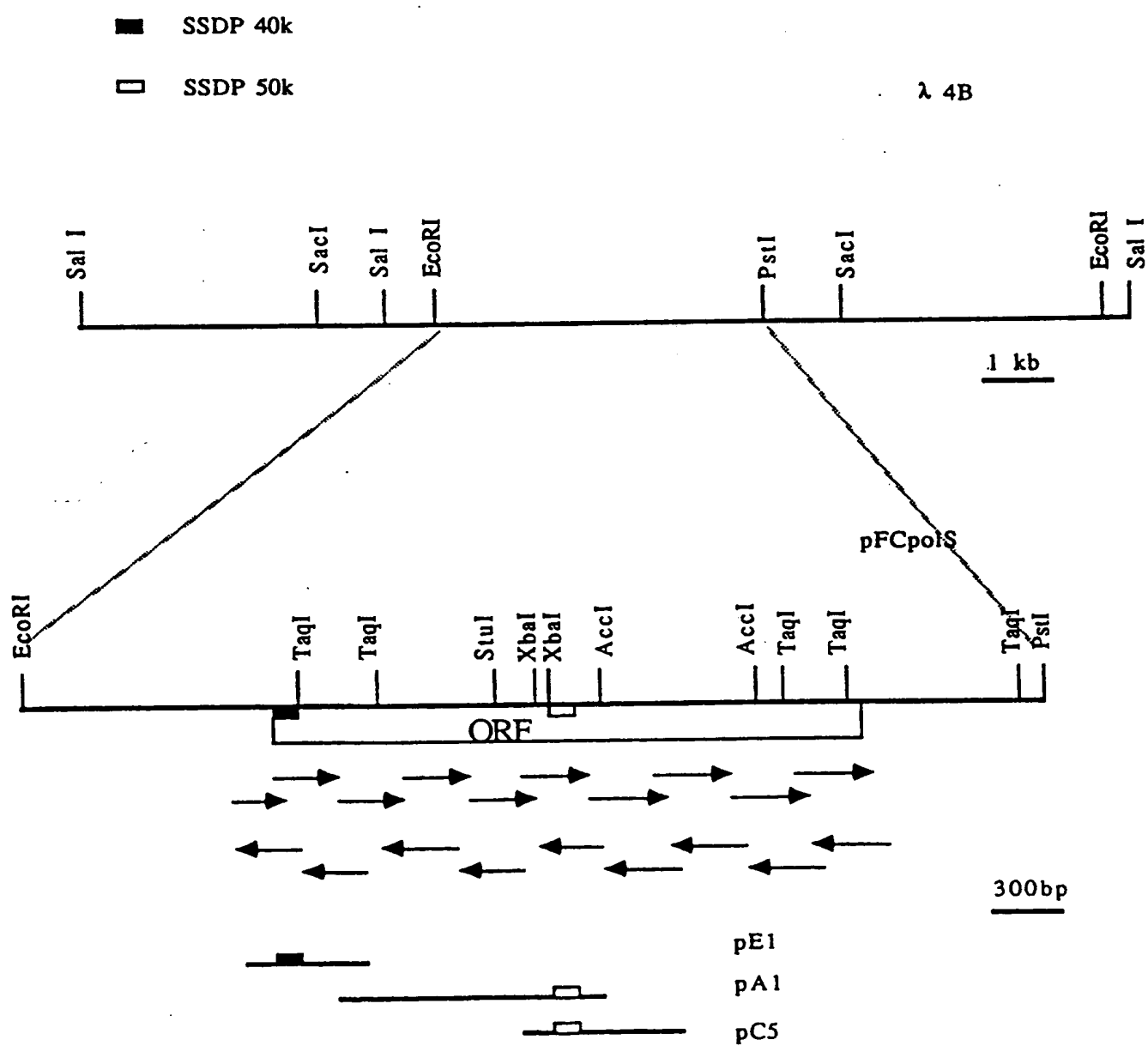


FIG. 2a

REGION 1		REGION 2		REGION 3		
Sso	227	RVAIDIEVYT	309	VLTFMGDDFDLPYIYFR	406	LIEYNFRDAEI
HSV	364	LMCFDIECKA	462	VIGYNIINFOWPFLAK	574	IGEYCIQDSL
ScII	286	VMAFDIETTK	374	ISTFMGDDFFDWPFFINNR	470	LSEYSVSDAVA
Ade02	137	FVTYDVEITYT	271	IVGHNINGFDEIVLAAQ	435	TLDYCALDVQV
VacV	137	YLFLDIECHF	234	VVTFMGHNFDLRYITNR	366	TGNVYVTVDEDI
T4	108	VANCDIEVTG	210	FTGWNIEGFDVPIIMNR	366	YISYNIIDVES
Ø29	8	MYSCDFETTT	58	LYFHNL-KFDGAFIINW	162	EYAYIKNDIQI
EcIIIe	8	QIVLDTETTG	95	LVIHNA-AFDIGFMDYE	149	CARY-SIDNSK
T7	1	MIVSQIEANA	56	IVFHMGHKYDVPALTKL	167	MMDYNVQDVVV
EcI	351	YFAFDTEIDS	416	KVGQNL-KYDRGILANY	494	AGRYAAEDADV
		*	*	*		*
REGION 4		REGION 5				
Sso	494	YKGA VVIDPP--AGIFFN-ITVLDFASLYPSIIIRTNLSYET-V				
HSV	696	YQGA KVLDP T--SGFHVNPVVVDFDFA SLYPSIIQAHNLCFST-L				
Ade02	522	IRGGRCYPTY--LGILREPLYVYD ICGMYASAL-THPMPWGPPL				
VacV	503	YEGGKVFA PKQ-KHFSNN-VLIFDYHSLYPNVCI FGNLSPET-L				
Hso	839	YA6GLVLDPK--VGFYDKFILLDFNSLYPSIIQEFNICFTT-V				
ScI	843	YQ6GLVFEPE--K6LHKNYVLVHDFNSLYPSIIQEFNICFTT-V				
EcII	398	SP6GYVMDSR--P6LYD-SVLVLDYKSLYPSIIIRTFIDPVG-L				
T4	388	FP6AFVFEPK---PIARRYIMSFDLTSLYPSIIIRQVNI SPETIR				
Ø29	226	YR6GFTWLNDRFKEKEIGEGMVFDVNSLYPAQMYSRLLPYGEPI				

REGION 5

Sso	594	LYDVVQRAMKVFIMATYGVVFGAETFP	LYAPRVAESVTALG-R
HSV	802	LLDKQQAIAIKVVCMNSVYGF	TGVQHGLLPCLHVAATVITIG-R
Ade02	686	KNQTLRSIAKLLSNALYGSFATKLDNKK	-IVFSDQMDAATLK
VacV	627	IYDSHQYTYKIVANSVYGLH6FRNSALYS	YASAKSCTSIG-R
Hsq	941	QYDIRQKALKLTANSMYGC	L6FSYSRIFYAKPLAALVTYK6-R
ScI	935	QCDIRQQAALKLTANSMYGC	L6GYVNSRIFYAKPLAALVTYK6-R
EcII	483	GNKPLSQALKIIMNAFYGV	L6TTACRFFDPRLASSITHRG-H
T4	548	LAMINQLNRKILINSLYGA	L6NIGHFRYYDLRNATAITIF6QV
429	374	SEGAIKQLAKLHLSLYG	KFASNPDVTGKVPYLKENGAL6FR

REGION 6

Sso	648	GLTVLYGDTDSLFLLNPPKNSLE	698 LKKNYFGVY
HSV	879	SHRIIYGDTSIFVLCRGLTAAG	937 AKKKYIGVI
Ade02	863	PLKSVYGDTSLSLFVTERGHR	LME 934 APKLYA-LK
VacV	719	RFRSVYGDTSVFTIIDSQOV	DK 777 SKKKYTTMK
Hsq	995	NLEVIYGDTSIMINTNSTNLEE	1051 KKKKYAALV
ScI	989	NLLVYVGDTSVMIOTGCDNYAD	1045 AKKKYAALV
EcII	537	G YDVIIYGDTSDFVWLKGAHSEE	612 SKKRYAGLI
T4	612	DF-IAAGDTSVYVCVK--VIE	701 AKKRYA-LN
429	449	YDRIIYCDTDSIHLTGTETIPDV-	490 KRAKY-LRQ

REGION 7

REGION 8

Sso	710	KVD-IKGM-LVKKRNTPE
HSV	949	KML-IKGVOLVRKNNCAF
Ade02	955	K-LRAK6HAA-EGLOYDT
VacV	799	R-I-NKGTSETRRDVSXF
Hs	1071	KQE-LKGLDIVRRONCOL
ScI	1965	VLE-VKGLDHKKRREFCPL
EcII	627	RHV-FKGLETVRTDWTPL
T4	721	H-LKIM6MET-QOSSTPK
429	512	K-LVE-6SPD-OYTQIKF

FIG. 2b

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IT 93/00058

## I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)<sup>6</sup>

According to International Patent Classification (IPC) or to both National Classification and IPC  
 Int.Cl. 5 C12N15/54; C12N15/63; C12N9/12

## II. FIELDS SEARCHED

Minimum Documentation Searched<sup>7</sup>

Classification System	Classification Symbols
Int.Cl. 5	C12N

Documentation Searched other than Minimum Documentation  
 to the Extent that such Documents are Included in the Fields Searched<sup>8</sup>

## III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup>

Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	EMBL Database Accession number X64466; 30 May 1992 ---	1-6, 8, 12-15
X	EP, A, 0 455 430 (NEW ENGLAND BIOLABS, INC) 6 November 1991 see page 4, line 3 - line 16; examples I-VI ---	1, 2, 5, 12-16, 18
X	THE ITALIAN JOURNAL OF BIOCHEMISTRY vol. 39, no. 2, April 1990, pages 83 - 99 R. RELLA ET AL. 'Purification and properties of a thermophilic and thermostable DNA polymerase from the Archaeobacterium Sulfolobus solfataricus' see page 83, paragraph 3 - page 84, paragraph 1 see page 94, paragraph 1 - paragraph 3; figures 7, 8 --- -/-	12-15

<sup>10</sup> Special categories of cited documents: <sup>10</sup>

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

13 SEPTEMBER 1993

Date of Mailing of this International Search Report

23. 09. 93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

MONTERO LOPEZ B.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	SYSTEM. APPL. MICROBIOL. vol. 7, 1986, pages 337 - 341 M. ROSSI ET AL. 'Struture and properties of a thermophilic and thermostable DNA polymerase isolated from Sulfolobus solfataricus' see abstract see page 340, left column, paragraph 2; figure 4 ---	12-15
P,X	NUCLEIC ACIDS RESEARCH. vol. 20, no. 11, 11 June 1992, ARLINGTON, VIRGINIA US pages 2711 - 2716 PISANI, F.M. ET AL. 'A DNA polymerase from the archaeon Sulfolobus solfataricus shows sequence similarity to family B DNA polymerases' -----	1-6,8, 12-18

IT 9300058  
SA 75960

**13/09/93**

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0455430	06-11-91	US-A- 5210036 JP-A- 5068547	11-05-93 23-03-93
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